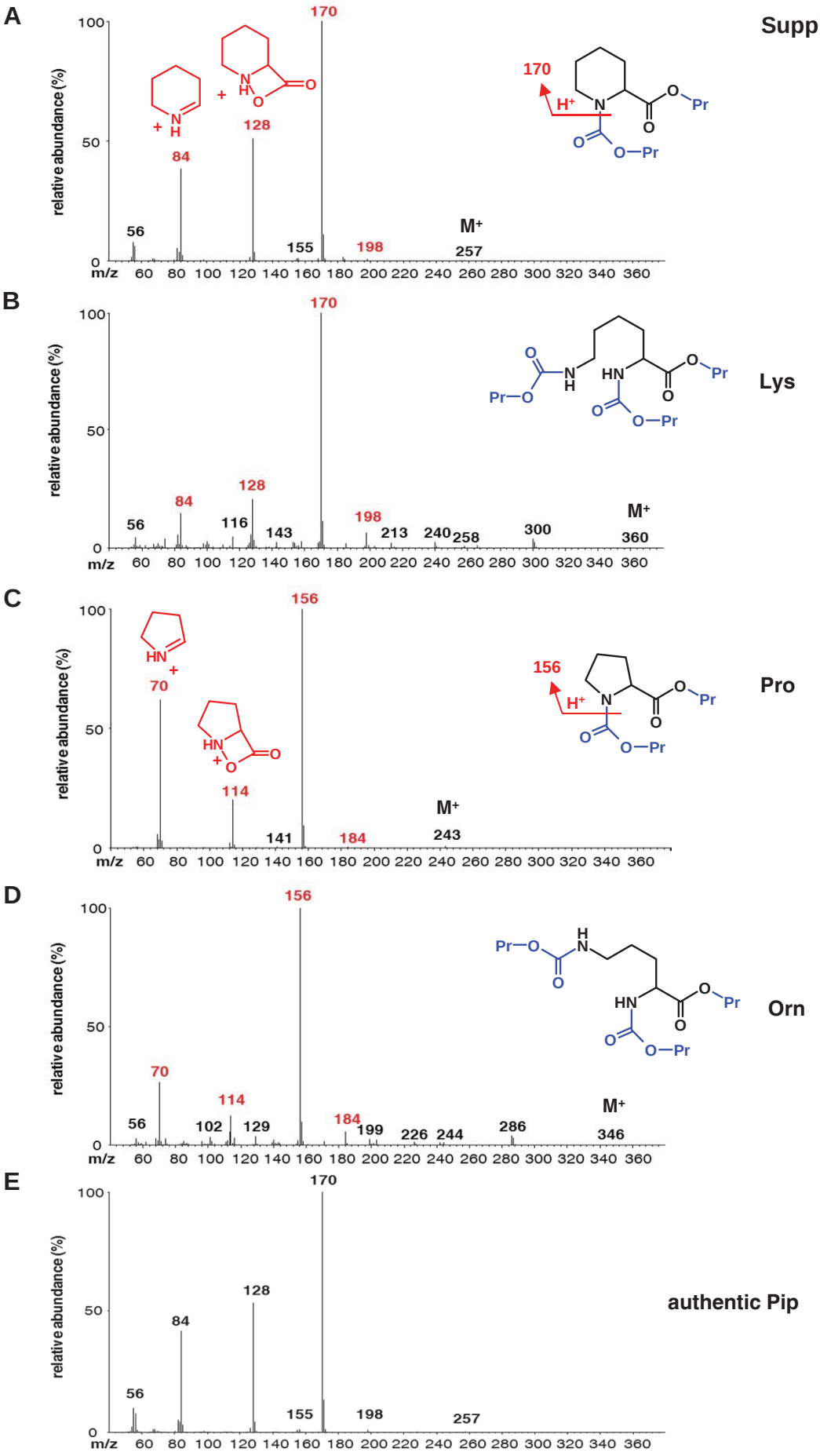
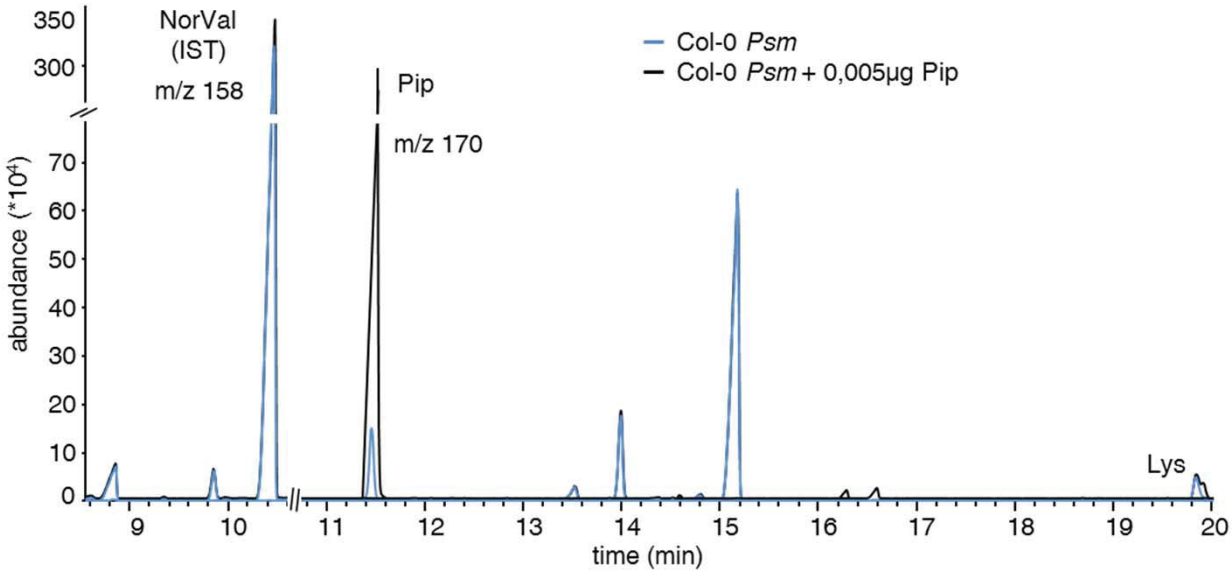
Published in "The Plant Cell doi: 10.1105/tpc.112.103564 , 2012"
which should be cited to refer to this work.

Supplemental Figure 1



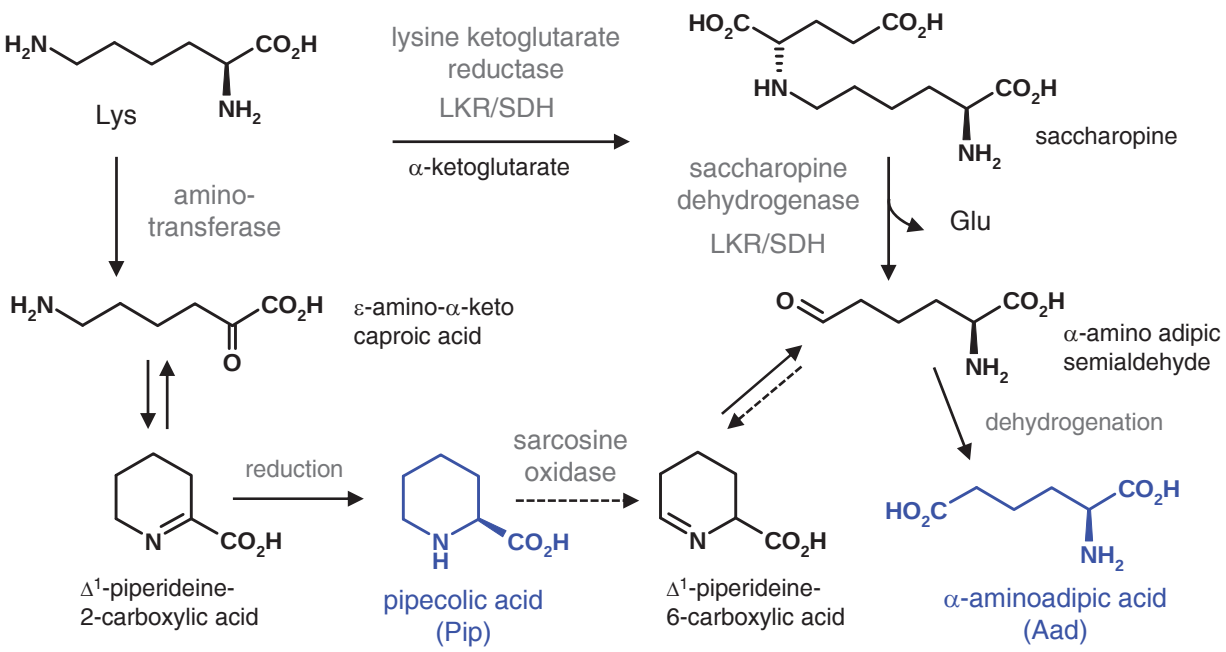
Supplemental Figure 1. Mass spectral identification of the initially unknown substance detected in extracts of *P. syringae*-inoculated plants as pipecolic acid.

(A) Mass spectrum of the unknown substance after derivatization with propyl chloroformate converting amino groups into propyl carbamate and carboxyl groups into propyl ester derivatives. The mass spectrum was not present in our mass spectral library containing 45 standard amino acids and amines. Containing the fragment series m/z 198, 170, 128, and 84, the spectrum showed similarities to the spectrum of the lysine derivative **(B)**. Moreover, the m/z 257 ion appeared to be the molecular ion (M^+) of the unknown substance. We recognized that a homologous relationship existed to the spectrum of the proline derivative **(C)** in a way that each of the fragments m/z 257 (M^+), 198, 170, 128, and 84 of the unknown substance spectrum (A) was replaced by a fragment reduced by 14 mass units: m/z 243 (M^+), 184, 156, 114, and 72, respectively (C). The spectra of the derivatives of lysine ("homoornithine") (B) and ornithine **(D)** exhibited a similar 14 mass unit fragment shift, which is consistent with the presence of an additional methylene group in lysine compared to ornithine. These observations and deduced structures of fragment ions (A, C) were consistent with the assumption that the unknown compound is pipecolic acid (homoproline), the methylene homologue of proline. **(E)** Authentic pipecolic acid yielded an identical mass spectrum than the extracted substance (A) after propyl chloroformate derivatization.



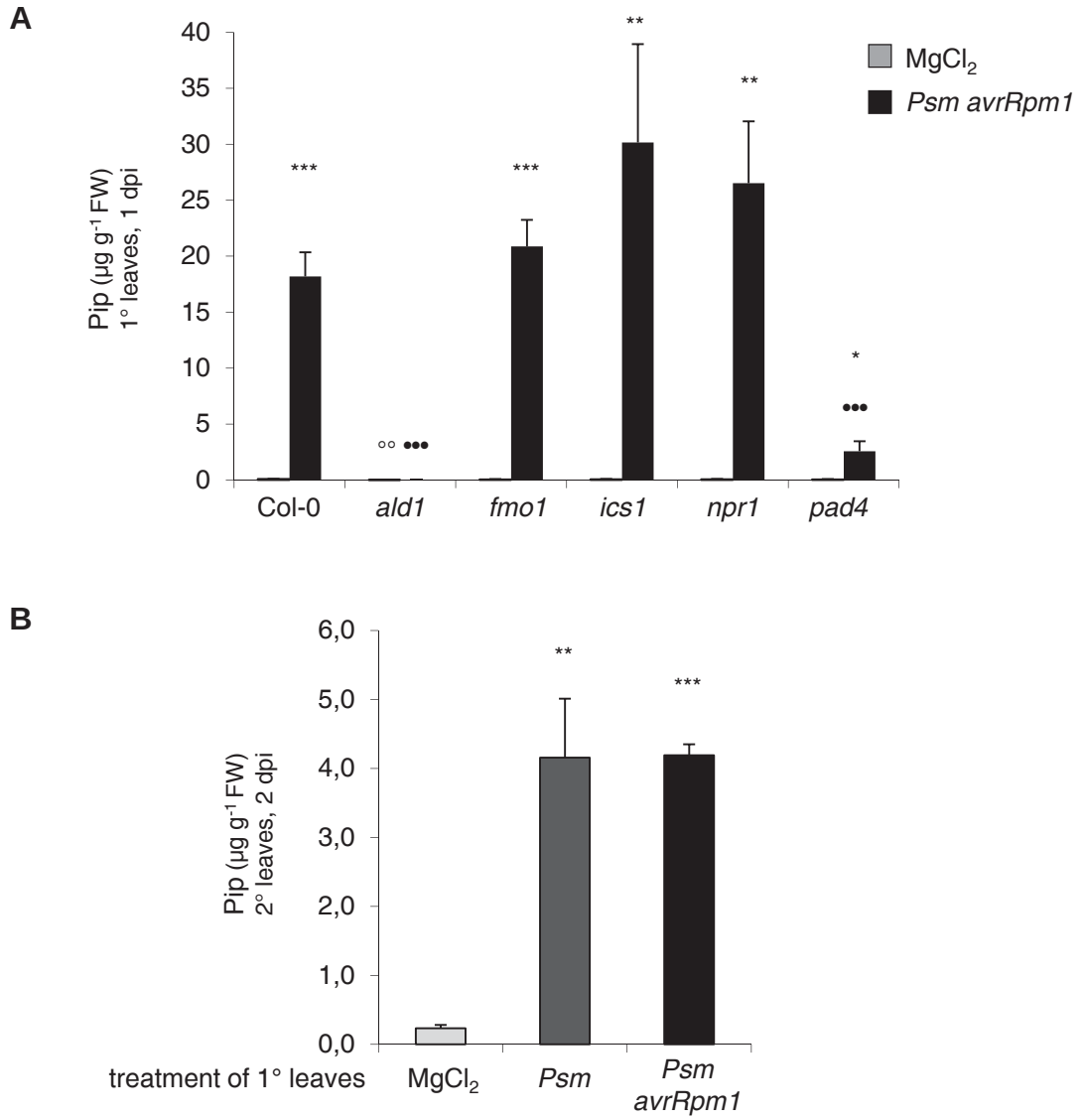
Supplemental Figure 2. The plant-derived substance identified as Pip and authentic Pip have identical GC retention times.

Overlay of GC ion chromatograms (m/z 158, before t = 10.6 min; m/z 170 after t = 10.6 min) derived from a pure plant extract sample (blue) and the same sample supplemented with 5 ng of authentic Pip demonstrate co-elution of extract peak and authentic Pip standard (retention time = 11.5 min). Note that NorVal (m/z 158, retention time = 10.4 min), which is used as an internal standard and was added to the plant extract before the work-up procedure, has similar abundance in the supplemented and original sample.



Supplemental Figure 3. Proposed scheme for pathogen-inducible Pip and Aad biosynthesis in plants via lysine catabolism.

The scheme is based on earlier (e.g. Galili et al., 2001; Gupta and Spenser, 1969; Song et al., 2004b; Goyer et al., 2004) and present findings (e.g. Fig. 3). Our presented findings demonstrate that the lysine aminotransferase ALD1 mediates pathogen-induced pipecolic acid biosynthesis. Whether ε-amino-α-ketocaproic acid and/or Δ1-piperideine-2-carboxylic acid are direct reaction products of an ALD1-catalysed transamination reaction still needs experimental verification.

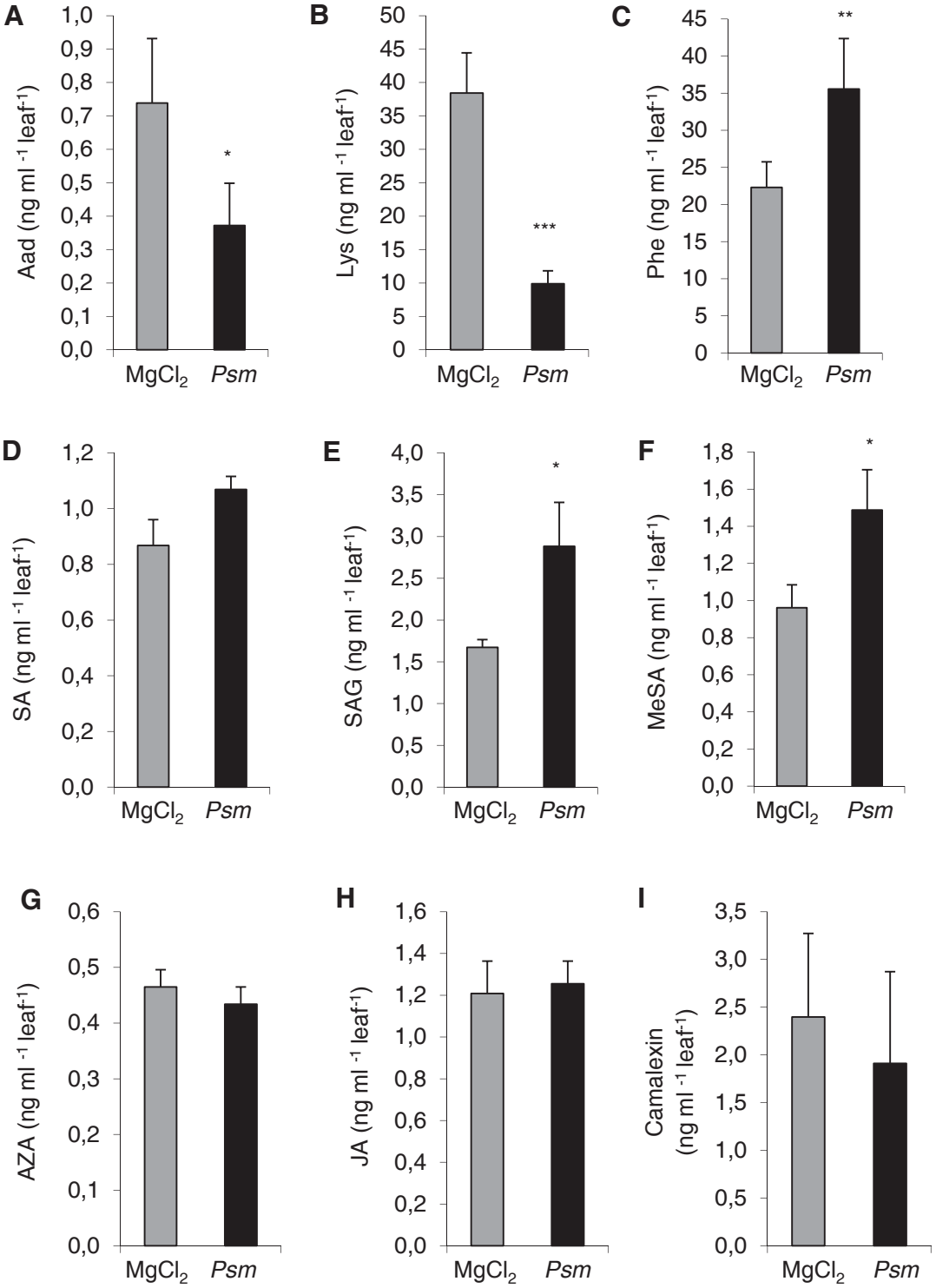


Supplemental Figure 4. *Psm avrRpm1*-induced Pip accumulation in inoculated and distal leaves.

(A) Accumulation of Pip in *Psm avrRpm1*-inoculated leaves of wild-type Col-0 and different defense mutant plants at 1 dpi. Details as described in the legend to Fig. 2.

(B) Pip accumulation in upper (2°) leaves following inoculation of lower (1°) leaves with *Psm* or *Psm avrRpm1* at 2 dpi. Details as described in the legend to Fig. 3.

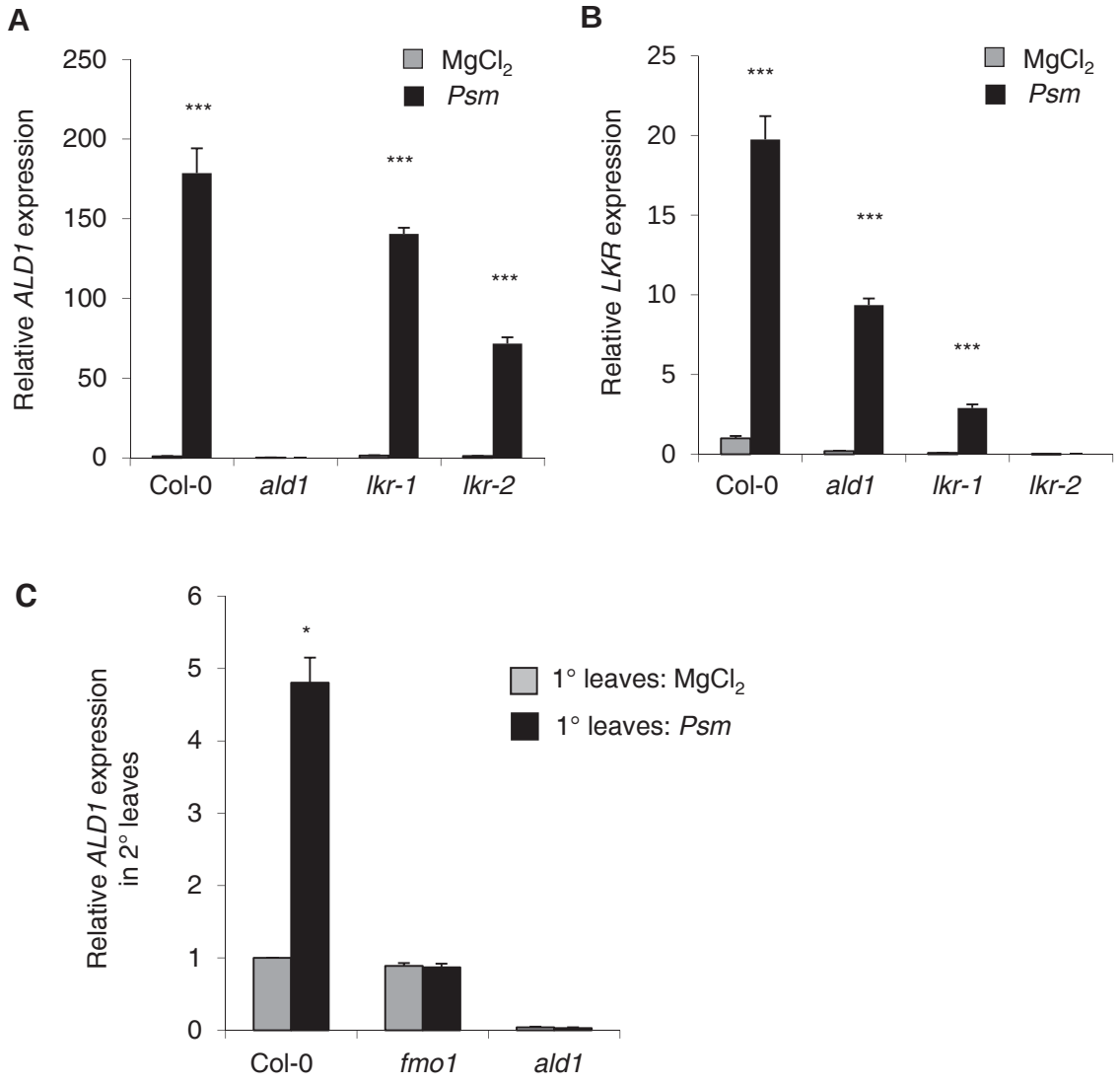
Supplemental Figure 5



Supplemental Figure 5. Metabolite levels in petiole exudates of leaves collected between 6 to 48 h post *Psm*- or $MgCl_2$-treatment.

Mean values are given in ng ml^{-1} exudate $leaf^{-1}$ ± SD from at least three replicate samples. Asterisks denote statistically significant differences between *P. syringae*- and $MgCl_2$-samples (***: P < 0.001; **: P < 0.01; *: P < 0.05; two-tailed *t* test). The corresponding values for Pip are depicted in Fig. 3D.

(A) α-Amino adipic acid (Aad). **(B)** Lysine. **(C)** Phenylalanine. **(D)** Free salicylic acid (SA). **(E)** Conjugated salicylic acid (SAG). **(F)** Methyl salicylate (MeSA). **(G)** Azelaic acid (AZA). **(H)** Jasmonic acid (JA). **(I)** Camalexin.



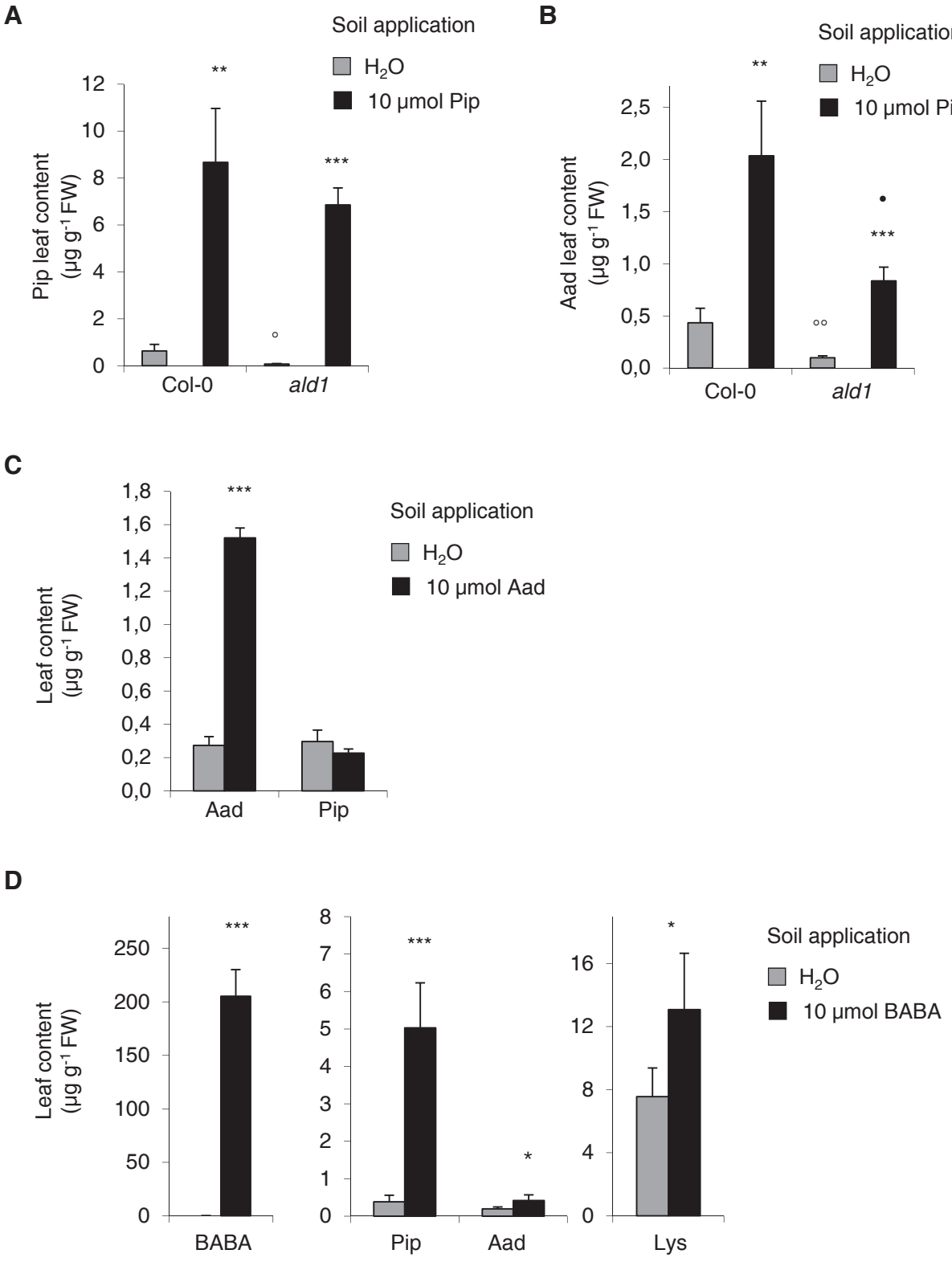
Supplemental Figure 6. Pathogen-induced *ALD1* and *LKR* expression in wild-type Col-0 and different mutant plants.

(A) and **(B)** *Psm*-induced *ALD1* (A) and *LKR* (B) expression in inoculated leaves of Col-0, *ald1*, *lkr-1* and *lkr-2* plants at 24 hpi.

(C) Relative expression of *ALD1* in upper (2°) leaves upon *Psm*-inoculation of lower (1°) leaves (2 dpi).

Transcript levels were assessed and data analyses were performed as described in the legend to Fig. 4A.

Supplemental Figure 7



Supplemental Figure 7. Metabolite levels in leaves following Pip, Aad, and β-amino butyric acid (BABA) application via the root in Col-0 and *ald1* plants.

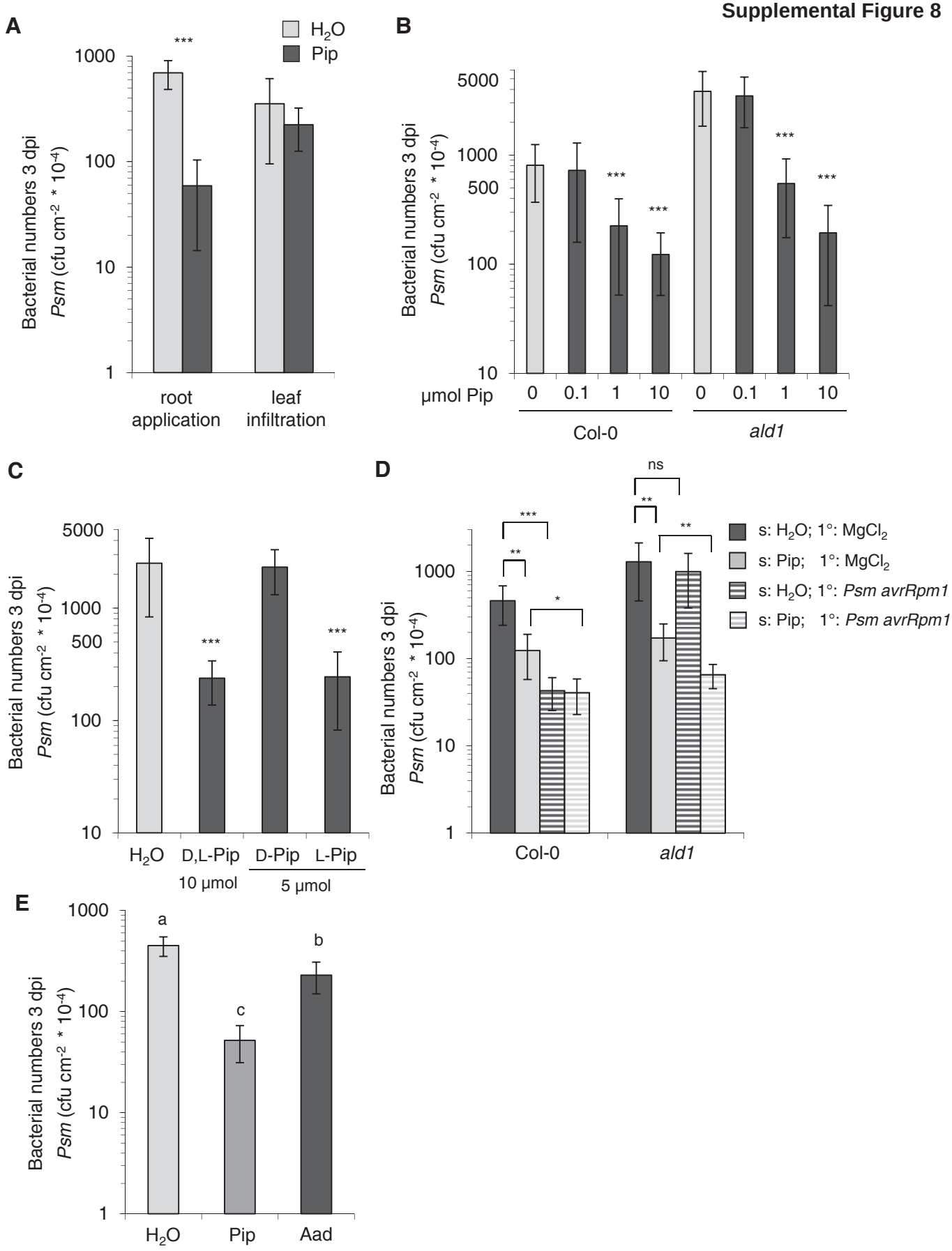
(A) and **(B)** Exogenous pipecolic acid supplied via the root is transported to the shoot and leads to an enhancement of Aad levels. Leaf contents of Pip (A) and Aad (B) in Col-0 and *ald1* plants one day after supplying 10 µmol Pip via the roots (as outlined in Fig. 5).

(C) Leaf contents of Aad and Pip in Col-0 plants one day after supplying 10 µmol Aad via the roots.

(D) BABA treatment induces Pip accumulation in Col-0 plants.

Leaf contents of BABA, Pip, Aad, and Lys one day after supplying plant pots with 10 µmol BABA. Mean values are given in µg g^{-1} fresh weight (FW) ± SD from at least three replicate samples. Asterisks denote statistically significant differences between samples from Pip-, Aad-, or BABA-fed and non-fed control plants (***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$, two-tailed *t* test). In (A) and (B), open (closed) circles indicate statistically significant differences of a H_2O- (Pip-) supplied *ald1* sample to the H_2O- (Pip-) supplied wild-type sample (two-tailed *t* test).

Supplemental Figure 8



Supplemental Figure 8. Concentration dependency of resistance induction by exogenous pipecolic acid, resistance-enhancing activity of L- but not D-Pip, and chemical complementation of *ald1* defects in *Psm avrRpm1*-induced SAR by exogenous Pip.

(A) Leaf resistance to *Psm* after application of 1 mM Pip via the root (as outlined in Fig. 5) or after infiltration into leaves. Bacterial inoculation was performed one day after Pip treatment.

(B) Concentration dependency of resistance induction by exogenous pipecolic acid in Col-0 and *ald1* plants.

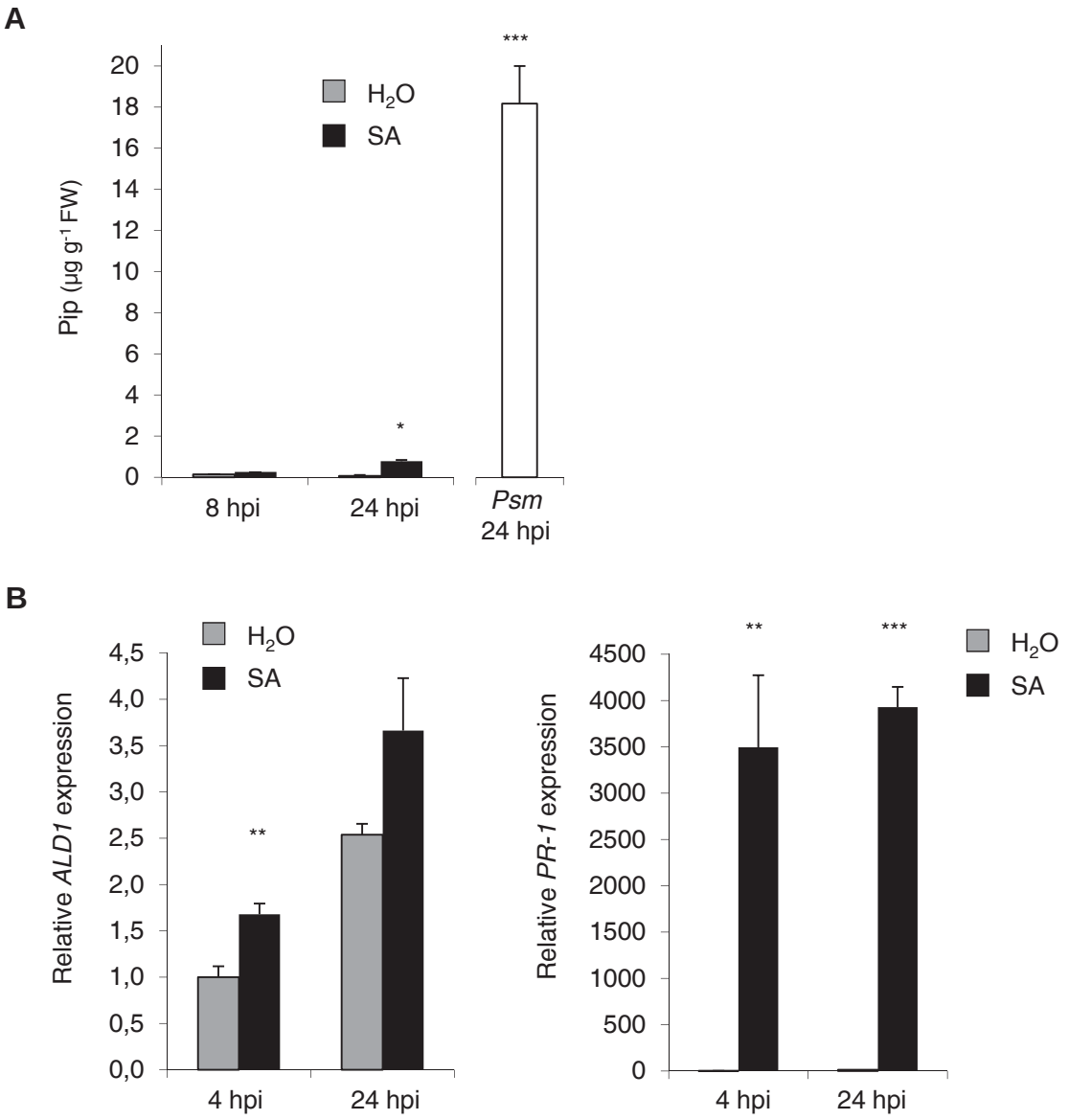
Plant pots were supplied with different amounts of D,L-Pip one day prior to inoculation. Experimental details were as outlined in the legend to Fig. 5. Asterisks denote statistically significant differences relative to the water control within each genotype (***: $P < 0.001$; two-tailed *t* test).

(C) Resistance-enhancing activity of L- but not D-Pip.

Indicated amounts of racemic D,L-Pip, D-Pip, or L-Pip were fed to plants one day prior to *Psm* inoculation, and bacterial numbers were scored at 3 dpi. Details as described in the legend to Fig. 5. Asterisks denote statistically significant differences relative to the water control (***: $P < 0.001$; two-tailed *t* test).

(D) *Psm* growth in upper leaves following SAR induction by *Psm avrRpm1* in lower leaves one day after exogenous Pip or water treatments via the root. Experimental details were as described in Fig. 6A, except that incompatible *Psm avrRpm1* instead of compatible *Psm* was used as the SAR-inducing pathogen.

(E) Col-0 resistance to *Psm* upon root application of water, 5 µmol L-Pip or 5 µmol L-Aad. Details as described in the legend to Fig. 5. Different letters above the bars denote statistically significant differences between pairwise compared samples ($P < 0.05$, two-tailed *t* test).



Supplemental Figure 9. Effect of exogenous SA on leaf Pip levels, *ALD1*-transcript levels, and *PR-1*-transcript levels.

(A) Pip levels in leaves at indicated times after infiltration with water, 0.5 mM SA, or a suspension of *Psm* (OD 0.005). Details as indicated in the legend to Fig. 2.

(B) Relative expression of *ALD1* and *PR-1* in Col-0 leaves at indicated times after infiltration with water or 0.5 mM SA. Transcript levels were assessed and data analyses were performed as described in the legend to Fig. 4A.

Primer name	Primer sequence (5' to 3')	Usage
ald1-fw	TTACGATGCATTTGCTATGACC	Genotyping of T-DNA lines
ald1-rv	TTTTAAATGGAACGCAAGGAG	Genotyping of T-DNA lines
lkr-1-fw	TCATTCTGCCTTCTCCATCAG	Genotyping of T-DNA lines
lkr-1-rv	AGCAACAACGATATTTCGTGG	Genotyping of T-DNA lines
lkr-2-fw	CGCTTCGATCATATCAAGAGC	Genotyping of T-DNA lines
lkr-2-rv	CCCCTATGACTTTCTGTGCAG	Genotyping of T-DNA lines
ALD1-FW	GTGCAAGATCCTACCTTCCCGGC	qRT-PCR
ALD1-RV	CGGTCCTTGGGGTCATAGCCAGA	qRT-PCR
LKR-FW	CATGTTGATGGGAAGAATCTC	qRT-PCR
LKR-RV	AATATCGTTGTTGCTTCGCT	qRT-PCR
FMO1-FW	TCTTCTGCGTGCCGTAGTTTC	qRT-PCR
FMO1-RV	CGCCATTTGACAAGAAGCATAG	qRT-PCR
PR-1-FW	GTGCTCTTGTTCTTCCCTCG	qRT-PCR
PR-1-RV	GCCTGGTTGTGAACCCTTAG	qRT-PCR
PTB-FW	GATCTGAATGTTAAGGCTTTTAGCG	qRT-PCR; reference gene
PTB-RV	GGCTTAGATCAGGAAGTGTATAGTCTCTG	qRT-PCR; reference gene

Supplemental Table 1. Primers used in this study.